

IN THE SPECIFICATION:

Please insert the sequence listing after the specification but before the claims.
On page 2 of the specification, please replace the paragraph beginning on line 35 with the following:

VEGF-A is a homodimeric glycoprotein formed by two 23-kDa subunits (Ferrara N, et al. Biochem Biophys Res Commun 165:198, 1989), of which five monomeric isoforms exist, derived from the differential splicing of the same RNA. These include two isoforms that remain ~~attach~~ attached to the cellular membrane (VEGF 189 and VEGF 206), and three of soluble nature (VEGF 121, VEGF 145, and VEGF 165). VEGF 165 is the most abundant isoform in mammal tissues, except for lung and heart, where VEGF 189 predominates (Neufeld G et al. Canc Met Rev 15:153, 1995), and in placenta, where VEGF 121 expression prevails (Shibuya MA et al. Adv Canc REs 67:281, 1995).

On page 6 of the specification, please replace the paragraph beginning on line 4 with the following:

Recently, two receptors ~~where~~ were identified for VEGF named NRP1 and NRP2. These belong to the neuropilins family (NRP), and act as co-receptors for some specific isoforms of proteins of the VEGF family: VEGF-A₁₄₅, VEGF-A₁₆₅, VEGF-B₁₆₇ and PlGF1, increasing their mitogenic capacity. The expression of NRP1 has become a marker of the aggressiveness of prostate cancer, has been related to the increase of angiogenesis in melanomas, and with apoptosis escape events in breast cancer (Latil A et al. Int J Cancer 89:167, 2000; Lacal PM J Invest Dermatol 115:1000, 2000; Bachelder RE Cancer Res 61:5736, 2001). The coordinate over-expression of NRP-1, KDR, and VEGF-A165 have been related to the fibrovascular proliferation in diabetic retinopathy cases and rheumatoid arthritis (Ishida S. et al. Invest Ophthalmol Vis Sci 41: 1649, 2000; Ikeda M. Et al. J Pathol 191:426, 2000).

NRP2 is over-expressed in osteosarcomas where it promotes angiogenesis and tumor growth (Handa A et al. Int J Oncol 17:291, 2000).

On page 7 of the specification, please replace the paragraph beginning on line 29 with the following:

In human tumors, VEGF is ~~over-express~~ over-expressed in the tumor compartment (Ferrara, No. Curr. Top. Microbiol. Immunol. 237:1, 1999), and high levels of VEGF and its receptors have been demonstrated in the tumor-associated vasculature (Brekken RA. J Control Release 74:173, 2001). The stromal cells also produce VEGF in response to the stimulus of transformed cells, with the result that when tumor cells are removed, VEGF levels persist in the patients. The presence of VEGF and its receptors have a practical value for the establishment of prognosis and staging in cases of prostate, cervix, and breast tumors (George DJ et al. Clin Cancer Res 7:1932, 2001; Dobbs SP et al. Br J Cancer 76:1410, 1997; Callagy G et al. Appl Immunohistochem Mol Morphol 8:104, 2000). On the other hand, VEGF is also within the group of soluble factors that, together with other cytokines like IL-10, TNF- α and TNF- β , (Ohm JE [[y]] & Carbone DP, Immunol Res 23:263, 2001), could be implicated in the immunosuppression that characterizes cancer patients (Staveley K, et al. Proc Natl Acad Sci USA 95:1178, 1998; Lee KH, et al. J Immunol 161:4183, 1998). This immunosuppressive effect seems to be related to its binding to the Flt1 receptor (Gabrilovich D et al. Blood 92:4150, 1998).

On page 8 of the specification, please replace the paragraph beginning on line 34 with the following:

Immunogens of polypeptidic nature of interest for the present invention, as well as their fragments, can be isolated from their natural sources or obtained by synthesis or recombinant DNA technology. These polypeptides can also be produced fused to

proteins with ~~acknowledged~~ having known adjuvant activity like ~~such as~~ p64K₁ (R. Silva et al US 5286484 [[y]] and EP 0474313), or ~~can be~~ covalently bound to ~~them~~ adjuvants after their individual obtainment following the polypeptide synthesis. Other available ~~strategy~~ strategies in these cases ~~is~~ are the obtainment of the natural polypeptide, its mutated or modified variants, and their fragments, as a part of loops exposed or not in bacterial proteins like OMP1, which are part of immunostimulatory preparations, in this particular case VSSP (R.Perez et al US 5788985 [[y]] and 6149921). Furthermore it is possible to obtain the polypeptidic immunogen exposed in the surface of a viral particle (HbsAg, VP2 of parvovirus, etc.), bound to specific peptides that target cells or organs specialized in the induction of the immune response (CTLA4, Fc segment of the Ig, etc.), or to proteins capable of increasing biodistribution like VP22.

On page 10 of the specification, please replace the paragraph beginning on line 2 with the following:

Additionally, the gene of interest can be preceded by the coding sequence for the mRNA replication machinery, in a way that mRNA is amplified in the target cell, increasing the expression of said gene, and with it, ~~of~~ the therapeutic/vaccine effect according to the invention. The replication machinery in question could be of alphavirus origin (Schlesinger S, Expert Opin Biol Ther. 1:177, 2001), more specifically derived from the Sindbis or Semliki viruses, or similar. In this particular case, the gene of interest is under the transcriptional control of a subgenomic promoter that allows the amplification of its mRNA in target cells, once the molecules according to the present invention have been internalized. ~~Besides-Furthermore~~, the DNA vector might contain sequences that permit the replication of the molecules, which are object objects of the present invention, in mammalian cells. This allows an increase in the expression levels and/or of the therapeutic/vaccine effect (Collings A., Vaccine 18:4601, 1999).

On page 15 of the specification, please replace the paragraph beginning on line 6 with the following:

The present invention has advantages over passive immunotherapy, which is in advanced phases of clinical trials using the same molecules as targets. In comparison with passive transfer of immunity through the administration of monoclonal antibodies (ex. Anti-VEGF), the immunization, be it with the protein or the oligonucleotide, has the advantage of inducing the ~~endogen~~ endogenous production of antibodies and in addition the proliferation and expansion of specific cytotoxic CD8+ lymphocytes.

On page 15 of the specification, please replace the paragraph beginning on line 25 with the following:

It is possible that the cytotoxic cellular response is mediated by the recognition of some of the peptides that appear in Tables 1 and 2. In these, some peptidic segments appear, that could be relevant in the cellular response directed to selected targets in the VEGF family, its receptors and co-receptors. This information was obtained through computer analyses on public databases from NIH and Heidelberg Institute (~~http://bimas.dart.nih.gov/molbio/bla_bind, and www.bmi-heidelberg.com/scripts/MHCServer.dll/home.htm~~) using BIMAS and SYFPEITHI software, respectively. The peptides marked and other sequences derived from the antigens of interest could be used for the active immunotherapy of the already described pathologies as a single or combined treatment, and as part or not of molecules with adjuvant capacities. These peptides can also be used in their oligonucleotide variants with vaccine purposes.

On page 20 of the specification, please replace the paragraph beginning on line 5 with the following:

VEGF isoforms were cloned applying the polymerase chain reaction (PCR) using as template a cDNA obtained from a previous isolation of mRNA of CaSki cell line (ATCC CRL 1550), according to the manufacturer instructions (Perkin-Elmer), and utilizing primers SEQ ID NO: 1 and SEQ ID NO: 2. Bands corresponding to the amplification products of VEGF isoforms 121 (SEQ ID NO: 19 and SEQ ID NO: 20), 165 and 189 were extracted from 2% agarose gels. After band digestion with endonucleases BamHI and ECORI, the cDNAs from the VEGF isoforms were purified and cloned independently in the vector (proprietary vector of CIGB). Resulting plasmids were sequenced and determined to have no mutations with respect to the amino acid sequences reported by the EMBL (~~www.embl-heidelberg.de~~) for the

cloned isoforms. The cDNA corresponding to VEGF isoforms were subsequently cloned KpnI/EcoRV on the pMAE5Δ5 vector, that among other characteristics differs from the pAECΔ2 by the presence of 5 immunostimulatory CpG sites.

On page 20 of the specification, please replace the paragraph beginning on line 21 with the following:

The mutated variant SEQ ID NO: 21 and SEQ ID NO: 22 was generated by PCR using the following primers:

(A) Amplification of the 5' terminal fragment (315bp): using primers with sequences SEQ ID NO: 3 and SEQ ID NO: 4

(B) Amplification of the 3' terminal fragment (93bp): using primers with sequences SEQ ID NO: 5 and SEQ ID NO: 6.

The fragments thus amplified were purified as referred, and were used in equimolar concentrations as a template for a fusion PCR using the primers corresponding to sequences SEQ ID NO: 7 and SEQ ID NO: 8. Resultant cDNA containing the mutation was digested BamHI/EcoRI, and was purified, and cloned in pAECΔ2 vector. The mutations introduced were checked by sequencing, and the DNA corresponding to VEGF_{KDR(-)} was subcloned KpnI/EcoRV in pMAE5Δ5 vector resulting in pMAE5Δ5 VEGF_{KDR(-)}.

On page 21 of the specification, please replace the paragraph beginning on line 9 with the following:

In the case of the extracellular domains 1 to 3 SEQ ID NO: 27 and SEQ ID NO: 28 (for domains 1-3) and SEQ ID NO: 29 and SEQ ID NO: 30 (for domain 3 alone), the primers used correspond to sequences SEQ ID NO: 9 and SEQ ID NO: 10. After digestion of the amplified fragment (943bp) SEQ ID NO: 25 and SEQ ID NO: 26 with endonucleases BamHI and EcoRI, the cDNA coding 1-3 domains of KDR was

purified, and cloned in pAECΔ2 vector. Clones positive by restriction analysis were verified by sequencing of the corresponding DNA. The cDNA corresponding to KDR 1-3 was then subcloned KpnI/EcoRV in the already described pMAE5Δ5 vector (pMAE5Δ5 KDR1-3).

For the cloning of transmembrane and cytosolic regions of the receptor a two-step strategy was designed. For the insertion of the first segment, the primers corresponding to SEQ ID NO: 11 and SEQ ID NO: 12 were used. After the XbaI/BglII digestion of this 747bp segment, the product was cloned in the pMAE5 vector, previously digested with the same enzymes, obtaining the plasmid pMAE5 KDR 747. This plasmid was digested BglII/NotI in order to insert the remaining carboxi-terminal fragment of 1091bp that was amplified using the primers corresponding to sequences SEQ ID NO: 13 and SEQ ID NO: 14. Clones positive by restriction analysis were verified by DNA sequencing and denominated pMAE5 KDR C.

On page 21 of the specification, please replace the paragraph beginning on line 27 with the following:

For the cloning of transmembrane and cytosolic regions of VEGF receptor (KDR) on the chickenpox virus, the primers corresponding to sequences SEQ ID NO: 15 and SEQ ID NO: 16 were used. After digesting this 953 bp segment with StuI/SmaI enzymes, the product was cloned in the pFP67xgpt vector, previously digested with the same enzymes. In this same vector, digested SmaI/BamHI, the remaining 919 bp were inserted, that were amplified from the original cDNA using primers corresponding to sequence SEQ ID NO: 17 and SEQ ID NO: 18. Clones positive by restriction analysis were verified by DNA sequencing and denominated pFP67xgpt KDR C.

On page 32 of the specification, please replace the paragraph beginning on line 28 with the following:

The analysis of the levels of murine VEGF (R&D kit for murine VEGF) in the sera of un-treated animals showed that with the increase of time of ~~exposal~~ exposure to tumor, the VEGF levels increased in serum, in concordance with the increase of tumor size with time. In the group immunized against human VEGF a significant reduction ($p < 0.001$ ANOVA, post-test Bonferroni) of murine VEGF levels was detected, that lasted past 30 days after the tumor challenge.